1925

REPRINT

BULLETIN OF THE EXPERIMENT STATION
HAWAIIAN SUGAR PLANTERS' ASSOCIATION

VOL. III BOTANICAL SERIES PART 1.

Morphological Studies of the Pythium-Like Fungi Associated With Root Rot in Hawaii.

BY C. W. CARPENTER

ISSUED AUGUST, 1921.

MORPHOLOGICAL STUDIES OF THE PYTHIUM-LIKE FUNGI ASSOCIATED WITH ROOT ROT IN HAWAII.

By C. W. Carpenter

Previous papers by the writer[1] have recorded the presence of a fungus of the *Pythium* type associated with root rot diseases of cane (Lahaina disease), pineapples (wilt), taro, rice, and bananas in Hawaii. Critical studies of the several strains isolated from the crops mentioned are being carried on with the object of determining the morphology of these organisms. Such studies, it is thought, may result in a better understanding of the nature of the diseases, the *modus operandi* of the causal agents and the relation of the environment as a limiting factor.

The pure culture of the fungus from cane, selected for the morphological studies chiefly to be discussed below, is the strain with which positive inoculations of cane and pineapple in pot culture have been obtained and previously reported. Other strains from cane, pineapple, and rice have been recently observed to have the same characters in general as the particular strain cited, while the strain from taro already reported as being parasitic differs in that it is a conidium-producing *Pythium*.

The cane fungus agrees very closely in both the asexual and the sexual stages with the sugar beet root rot fungus *Rheosporangium aphanodermatus* first described by Edson.[2] Such a fungus, which in a single trial was not found to be parasitic, is recorded in the writer's most recent paper.[3] Several additional strains of this sort of fungus, besides the one particularly discussed herein, have been isolated from cane and pineapples.

Edson notes the resemblance of his fungus to *Pythium* in the following language:

> "In the general character of the disease produced in seedlings and in its appearance in culture, the organism resembles *Pythium debaryanum* so closely as to be readily confused with it, except in the asexual fruiting stage.
>
> "The disease which it produces on the sugar beet is very similar to that caused by *P. debaryanum*. The fungus is even more aggressive as a parasite than *Pythium*. . . . . A disease of the side roots of growing beets was encountered during the course of the studies in soils which had been inoculated with artificial cultures of the fungus several

---

1 Carpenter, C. W., Hawaii Agric. Exper. Sta. Annual Report, 1918, p. 43 (rice root rot), p. 44 (taro rot). Report 1919, p. 50.
Preliminary report on root rot in Hawaii. Hawaii Agric. Exper. Sta. Press Bul. 54.
Pythium in relation to Lahaina disease and pineapple wilt. *In* Hawaiian Planters' Record Vol. XXIII, p. 142–174, 1920.

2 Edson, H. A., *Rheosporangium aphanodermatus*, a new genus and species of fungus parasitic on sugar beets and radishes. *In* Jour. Agr. Res. Vol. IV, No. 4, 1915, p. 279–291. 5 Pls. See also Vol. IV, No. 2, p. 161–163.

3 Hawaiian Planters' Record, Vol. XXIII, p. 167, 1920.

months earlier. The fungus was readily isolated from diseased side roots of this beet and there appears to be no reason to doubt its causal relation to the trouble.

"The causal relation of the organism to the radish disease[1] as well as to damping-off of sugar beet seedlings was confirmed repeatedly by inoculation experiments."

That the cane fungus should be identical with the sugar beet fungus is rather improbable in view of the lack of close botanical relationship of the hosts. Similarly it would be rather surprising if the several strains from such botanically diverse crops as cane, pineapples, rice and bananas should be identical. A description of the parasitic strain from cane and a comparison with *Rheosporangium aphanodermatus* follows. Some figures (Pl. 23, figs. 7–10) of the taro rot organism previously reported as parasitic are also given by way of illustrating the reduced diplanetism in *Pythium*. Studies of these Phycomycetes associated with the root rot problem will be continued.

The Cane Pythium in Pure Culture.

The cane fungus grows well in pure culture on several media, with an abundant white, fluffy, aerial mycelium in evidence after a few days. In petri dish cultures the growth is for the first few days restricted to the surface film, but it produces a copious white aerial mycelium after becoming established on the substratum. In slant cultures the mycelium grows up in the tube often filling the bore for some distance above the top of the slant. After some weeks it settles back as a gelatinous, tough layer on the medium.

Media found satisfactory for the isolation and culture of this fungus are as follows: For isolation, an agar medium more dilute than standard; bouillon 1 part, distilled water 4 parts, plus 1% dextrose and 2% agar. An oat agar similar to Clinton's, made from rolled oats, is satisfactory for the development of the sexual spores. For studies of asexual sporulation Lahaina cane cuttings were disinfected in mercury bichloride 1-1000 for four minutes, rinsed in sterile water and allowed to produce roots in sterile water. Such young roots (about 1 inch long) treated with bichloride for 2 minutes, rinsed, and placed in sterile water in petri dishes, when inoculated furnished good material for the microscopic studies of the asexual stage.

Young hyphae are 2.8 to 7.0 μ in diameter, non-septate; in plate cultures they often show a peculiar twisting of the short branches, which gives a knob-like appearance under the microscope (Pl. 16, fig. 1); the hyphae are septate in age and in fructification.

Sexual Stage.

On oat agar oogonia develop in 3 to 10 days and are rather characteristically entangled in the antheridia of which there may be several with their supporting branches surrounding the oogonium. The oogonia (Pl. 19, fig. 2; Pl. 23, figs. 1–6) are terminal, 24.0–35.0 μ in diameter, the average of 50 being 29.0 μ.

[1] Black root disease.

Antherida are often from the same branch as the oogonium. The oospores are round, smooth, thick walled, slightly brownish in maturity, 21.0–28.0 μ in diameter, the average of 50 being 24.0 μ. In cane roots from the field the vestiges of the oogonium are 20.0–26.0 μ in diameter while the oospores are 18.0–24.0 μ, averaging 20.5 μ in diameter. In cane roots from plants inoculated with pure culture the oogonia measure 21.0–30.0 μ, and the oospores 20.0–25.0 μ, with an average of 22.0 μ.

Asexual Stage.

In water cultures the fungus is found to have invaded and occupied the cells of the young cane roots in a few days, the appearance (Pls. 20–21) being exactly similar to what we frequently find in field material.

Irregular globular or swollen bodies (presporangia), walled off at the tips of the hyphae, occur on agar plates (Pl. 18, fig. 1) rather infrequently, but in water culture they are formed in abundance (Pl. 20, fig. 1). They occur in the water near the surface of the root, and similar swollen bodies also fill numbers of adjacent root cells (Pl. 21, fig. 1). Resting spores are likewise formed abundantly in the cells of the cane root in water cultures.

The presporangia (Pl. 18, fig. 1; Pl. 22, figs. 1–4) vary greatly in size and shape, but they generally consist of several closely attached spherical masses, densely and finely granular and slightly brownish in color. The portions are characteristically more globose than represented by Edson for *Rheosporangium aphanodermatus*. If the water is frequently changed by means of a sterile pipette, some of the presporangia can be induced to develop in a few hours (Pls. 18–19). The contents of the presporangia flow out through a long, narrow, tubular process,[1] forming a delicate, spherical body in which the zoospores are differentiated. This is the true sporangium, if we follow Edson. The presporangia, when emptied, are scarcely to be seen, so thin are their walls, and so completely are their contents drawn out to form the sporangium.

The sporangia are spherical, dense, protoplasmic masses varying greatly in size. Their size seems to be determined by the size of the presporangial mass. As cleavage begins, the thin, hyalin, scarcely visible wall of the sporangium may be seen surrounding the contents at a distance of about 15.0 μ.

Streaming of the presporangium to form the sporangium in one case occupied eight minutes; a rocking movement began four minutes later, with cleavage lines well defined twelve minutes subsequently. Individual movement of the zoospores began in another two minutes. The zoospores were liberated in eight minutes, or thirty-four minutes from the formation of the sporangium. Another case similar to many which have been seen is sketched in Plate 22.

Of ten sporangia the average diameter of the contents was 48.0 μ and, of the hyalin sporangium, 65.0 μ. The number of zoospores varies from four to over a hundred estimated; generally in culture the number was eight to twenty-four estimated; the average size of zoospores being 10.0–12.5 x 7.5–

1 Considerably longer proportionately than shown in Pl. 22, fig. 5.

9.5 μ. Two cilia are attached at the sinus. After rounding up, the zoospores have been observed to germinate in the course of an hour, with a single germ tube. Under suitable conditions, this grows out into a hypha capable, conceivably, of attacking roots.

The fungous hyphae extend themselves through the cells of the young root, apparently being able to enter the root and penetrate the cell walls without difficulty. Although the fungus can at times be seen to grow intercellularly, it is generally to be found within the cells. After a short time (a few days) the mycelium has appropriated the cell contents more or less completely and to a similar degree occupied the interior of the cell, often being tightly packed therein. This is accompanied by the development of the hyphae into swollen, globular, thin walled protoplasmic masses, which the writer considers to be presporangia.

The resting spores are formed generally later than the zoospores in artificial cultures, and this appears to be the general rule in the roots, though the conditions of the environment rather than the mere duration of time determine the sequence.

Judging from observations of this cane fungus in the roots and in culture, the life cycle of the fungus in the field may be outlined by analogy, as follows:

In a cane root in wet soil, after the mycelium has developed as above outlined, zoospore formation is the rule, the mycelium emptying into the presporangia, which in turn empty into the sporangia, and scatter as zoospores. It is considered probable that the tube of delivery of the presporangium is capable of penetrating the cell walls, thus liberating the zoospores in the water outside the root. The empty mycelium and inconspicuous, empty presporangia remain as evidence of the cause of the disease. No traces of the sporangium are to be seen after the liberation of the zoospores. Under less favorable conditions (drier soil?), the fungus forms the relatively conspicuous oospores in the root cells. It is thought that if this is indeed the train of developments as they occur in the field, our frequent inability to find the fungus present in sufficient abundance to account for the damage to the root system of cane and pineapples is in considerable measure explained.

Pythiaceae and the Genus Rheosporangium Edson.

Edson considered his sugar beet fungus to belong in the *Saprolegniaceae*. He used the classification of Minden,[1] and finding no appropriate genus of the *Saprolegniaceae* to accommodate his fungus, established a new genus, *Rheosporangium*. Although the present writer has not been able to consult Minden's classification, he doubts the wisdom of this course for the reasons given below. It seems more natural to include this fungus under the genus *Pythium* of the *Pythiaceae*, whether we consider the latter as allied more closely to the *Saprolegniaceae* or to the *Peronosporaceae*.

---

[1] Minden, M. D. von. Pilze. *In* Kryptogamenflora der Mark Brandenburg, Bd. 5. Heft 3–4, 1911–12.

For a clear presentation of Edson's point of view the author quotes the following paragraph:[1]

> "The fungus under consideration seems to present a third and hitherto undescribed type of diplanetism, in which the first mobile period consists in the migration of the entire uncleaved sporangium and its contents from the presporangium. This type of egress is new. In all related forms previously described the spores are differentiated before migration. This distinction seems sufficiently important to justify its recognition as of generic rank. The uncleaved protoplasm rather than the differentiated spore migrates."

It seems to the writer that the type of reduced diplanetism Edson describes is essentially what we have throughout the group *Pythium.*[2] In *Pythium,* according to Butler's[3] description of *P. debaryanum,* we have a sporangium which might well be called a presporangium since the contents flow out undifferentiated through a beak, the differentiation taking place in a thin walled vesicle. (Cf. Pl. 23, fig. 10.) This primary migration is not essentially different from that in *Rheosporangium.* The wall of this vesicle in the latter genus is the wall of the protoplast which is delivered and draws out with it the contents of the sporangium. Butler[4] gives the origin of this vesicle in *Pythium* as the thin membrane at the tip of the beak, for he says (p. 34): . . . . . "the beak blows up into its vesicle." (p. 28): "The beak itself is a protrusion of the endosporal lining of the sporangial wall." Butler made a general survey of the group, and considered the phylogenetic relationship therein. He refers several times to the migration of the uncleaved sporangium. In speaking of the relation of *Pythium* to the *Saprolegniaceae,* he says (p. 50):

> "As far as the thallus is concerned, and in certain of the cruder details of reproduction the aquatic species of *Pythium* approach *Saprolegniaceae,* particularly *Pythiopsis* and to a lesser degree *Aphanomyces.* But these resemblances are superficial and are probably partly connected with the environment. In the essential points in which affinities must be looked for, the divergence is considerable. *Thus the zoospores are never liberated in a bladder as always occurs in Pythium.*[5]

And further on, in speaking of the affinity between *Pythium* and the *Peronosporaceae:*

> "But the genus *Pythium* is separated from all the rest by liberating its zoospores in an imperfectly differentiated state into a bladder at the mouth of the sporange, in which differentiation is completed."

---

1 Jour. Agr. Res. Vol. IV, No. 4, p. 290, 1915.

2 See Engler, A., and Prantl, K. Die Naturlichen Pflanzenfamilien. Teil I. Abt. I, p. 104.

3 Butler, E. J. *Pythium debaryanum* Hesse. *In* Memoirs of the Dept. of Agr. India, Vol. V, p. 262–266, 1913.

4 Butler, E. J. An account of the genus Pythium and some Chytridiaceae. *In* Memoirs Dept. Agr. India, Vol. I, No. 5, p. 1–158, 1907.

5 Italics, the writer's.

The fungus agrees rather closely with the species of *Pythium* which Butler[1] has described and provisionally called *P. gracile* Schenk. Subramaniam[2] has recently described such a fungus which differs from Butler's *P. gracile* in not being an algal parasite. He says in part:

> "The mycelium is composed of much branched hyphae, sometimes showing false dichotomy, very variable in breadth, from 3.0 to 8.0 μ. Septation occurs very irregularly in old cultures. *Irregular swellings on the hyphae were quite common*[3] when grown in plant tissues or in water cultures. . . . . *The irregular swellings on the hyphae serve as reservoirs of protoplasm and under favorable conditions sporangia may develop from them. It is not uncommon to find empty cells at the base of the sporangial hyphae.*[3]

Subramaniam considers this a new species and names it *P. Butleri.* The close similarity of this species to the cane fungus is best shown by quoting his description *verbatim:*

> *"Pythium Butleri n. sp.* (Subramaniam).
>
> "Mycelium composed of much branched hyphae, sometimes showing false dichotomy, the main strands being 3 to 8 μ broad and the lateral ramifications thinner. Irregular swellings quite common on the mycelium, which is septate in old stages. Sporangia lateral, elongated, slightly swollen at the tip. Zoospores few to 35 in number, bean-shaped, bi-ciliate, measuring when moving 8 to 12 μ, 6 to 8 μ in diameter, and after coming to rest 7 to 11 μ. Oogonia lateral or intercalar, spherical or subspherical, thin walled, and measuring 18 to 33 μ (average 26 μ). Antheridia terminal or intercalar (when they are usually on a different hypha from that bearing the oogonium), or hypogynal (when they are on the same hypha as the oogonium), knob-shaped. Oospores round, smooth, hyaline or light yellowish when fully mature, thick-walled, never filling the oogonium completely, 13.5 to 25.3 μ in diameter (average 21 μ). Oospores germinate by a germ tube, not by zoospores. Parasitic on *Nicotiana Tabacum, Zingiber officinale, Carica Papaya, Capsicum annuum,* and capable of attacking, when artificially inoculated, *Solanum tuberosum* and *Ricinus communis."*

Some data on the fungi *Rheosporangium aphanodermatus* Edson, *Pythium Butleri* Subramaniam and the cane *Pythium* are tabulated for convenience of comparison. The writer considers the cane fungus morphologically the same as *R. aphanodermatus* and *P. Butleri.*

---

[1] Butler, E. J. *In* Mem. Dept. Agr. India, Vol. I, No. 5, p. 67-71. 1907.

[2] Subramaniam. L. S. A Pythium disease of ginger, tobacco, and papaya. *In* Mem. Dept. Agr. India, Vol. X, No. 4, p. 181–194 (6 Pls.), 1919.

[3] Italics, the writer's.

TABLE I

Comparison of certain characters of *Rheosporangium aphanodermatus*, *Pythium Butleri*, and the cane *Pythium*.

	Young Hyphae	Zoospores	Oogonia		Oospores	
			Limits	Average	Limits	Average
R. aphanodermatus ..	2.8–7.3 μ	12.0x7.5 μ	22.0–27.0 μ		17.0–19.0 μ	
P. Butleri	3.0–8.0 μ	8.0–12.0x6.0–8.0 μ	18.0–33.0 μ	26.0 μ	13.5–25.3 μ	21.0 μ
Cane *Pythium*	2.8–7.0 μ	10.0–12.0x7.5–9.5 μ	24.0–35.0 μ (Culture)	29.0 μ	21.0–28.0 μ	24.0 μ
			20.0–26.0 μ (Cane root from field)		18.0–24.0 μ	20.5 μ
			21.0–30.0 μ (Cane root in water culture)		20.0–25.0 μ	22.0 μ

Summary.

1. The Pythium-like fungus previously reported as an active factor in the root rot disease of cane (Lahaina disease) is morphologically identical with *Rheosporangium aphanodermatus* Edson and *Pythium Butleri* Subramaniam.

2. The Pythium-like fungus previously reported as associated with the root rot of pineapples (wilt) and rice is similar in its morphology to the cane Pythium.

3. A taro rot fungus previously reported as like Pythium has been found to be a conidium-producing Pythium.

4. The writer considers that the cane fungus manifests a type of diplanetism in the asexual stage allied to that in the conidium-producing Pythiums, and prefers to classify it in the genus *Pythium* rather than in Edson's new genus *Rheosporangium*.

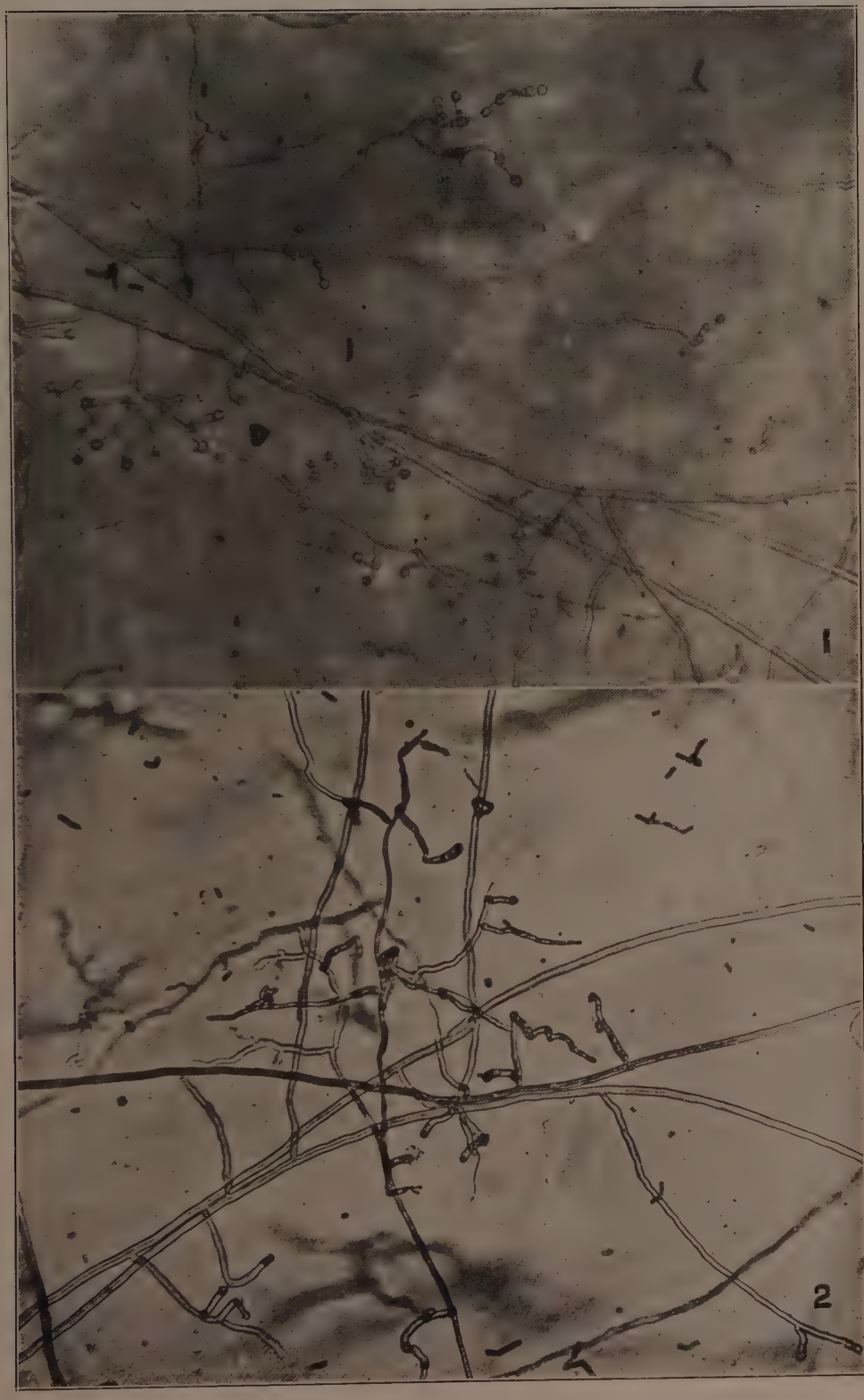
EXPLANATION OF PLATE 16.

Characteristic habit growth of young hyphae of *Pythium Butleri* from cane and pineapples. Note the short knobbed branches which suggest spores. The tips are not walled off, however. × 200.

Figure 1—*P. Butleri* from cane, isolated from natural root infection of Lahaina cane plant in sick Waipio soil, pot culture.

Figure 2—*P. Butleri* from pineapple. Isolated from collapsed roots of a plant from Kailua, Oahu.

PLATE 16.



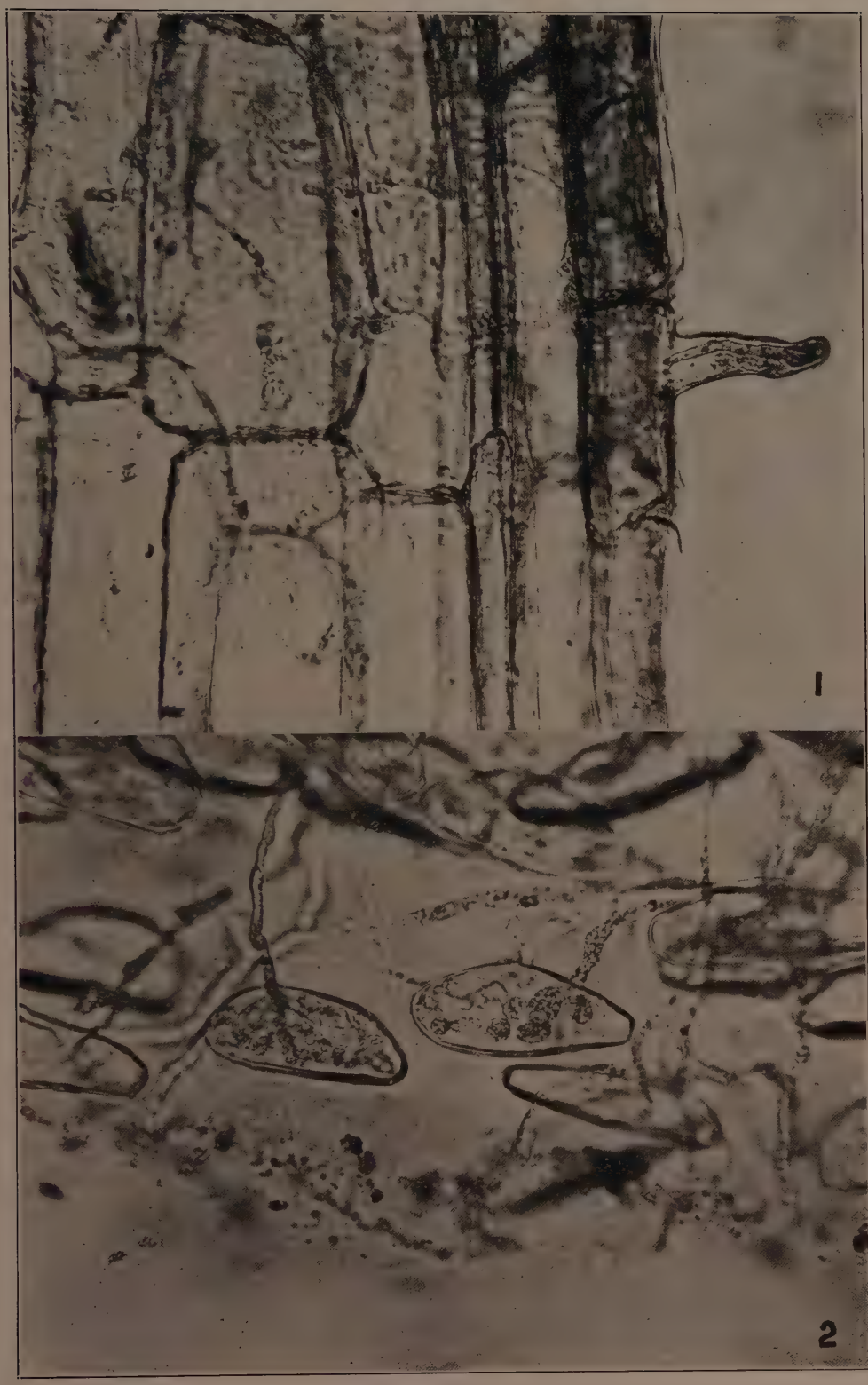
EXPLANATION OF PLATE 17.

Pythium Butleri in root cells.

Figure 1—Section of Lahaina cane root from Waipio. Root cells and young root-hair showing mycelium of the *Pythium* type. × 500.

Figure 2—Cells of root-cap occupied by the same sort of mycelium. × 500.

PLATE 17.



EXPLANATION OF PLATE 18.

Features of life cycle of *Pythium Butleri* from cane. Pure culture studies.

Figure 1—Mycelium and presporangia, water culture from oat agar plates. × 200.

Figure 2—The emptied presporangium and the dark sporangia; the darker sporangium has just completed its streaming from the presporangium. Water culture, cane root. × 200.

Figure 3—Zoospores in hyalin zoosporangium inactivated with osmic acid. Water culture, cane root. × 200.

PLATE 18.

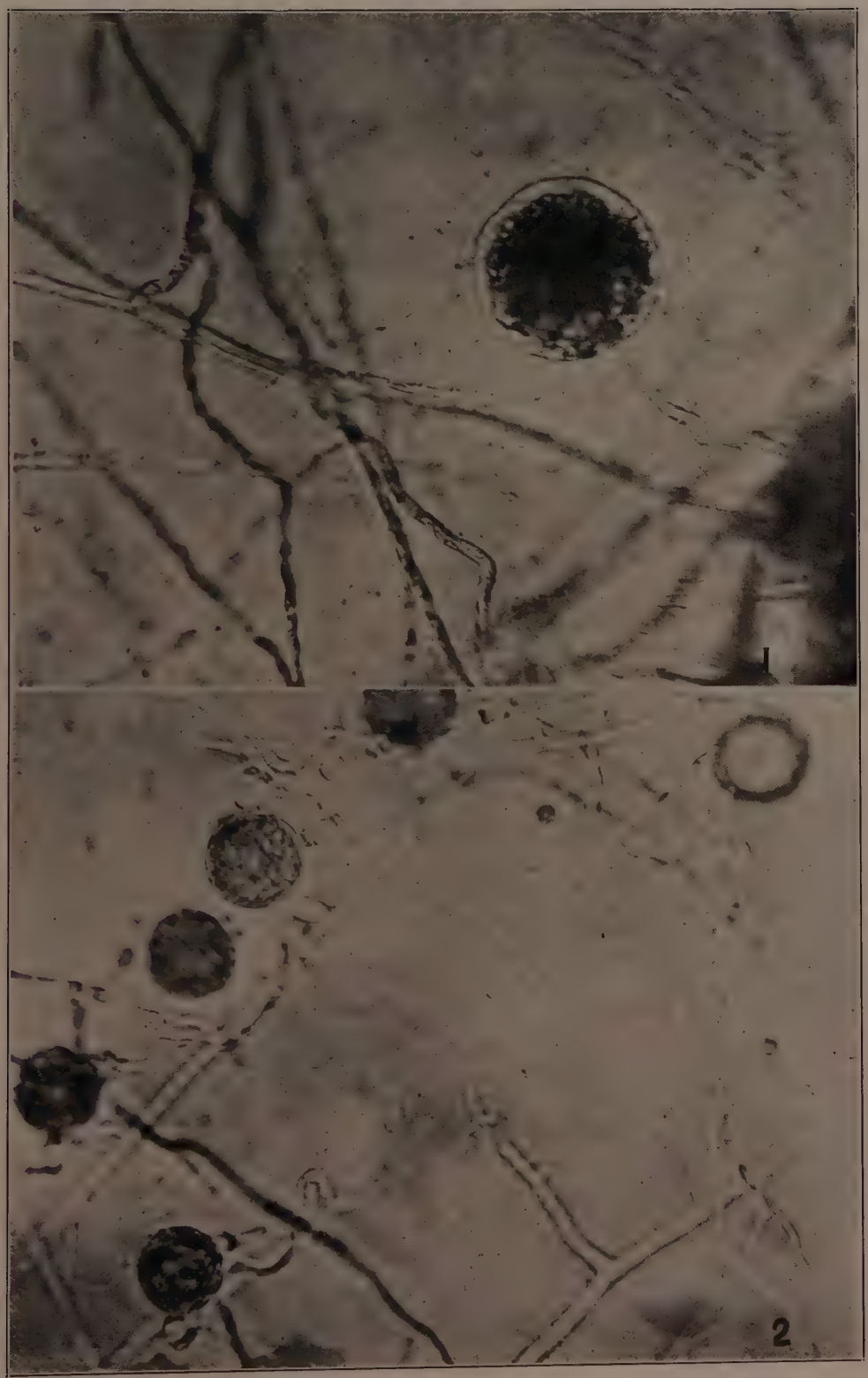
EXPLANATION OF PLATE 19.

Pure culture life cycle studies of *Pythium Butleri* from cane (continued).

Figure 1—Maturing zoosporangium with its hyalin wall, more highly magnified. Cleavage lines becoming evident. × 500.

Figure 2—Oospores developing. Note the several antheridia about one oogonium. A few days old oat agar culture. × 500.

PLATE 19.



EXPLANATION OF PLATE 20.

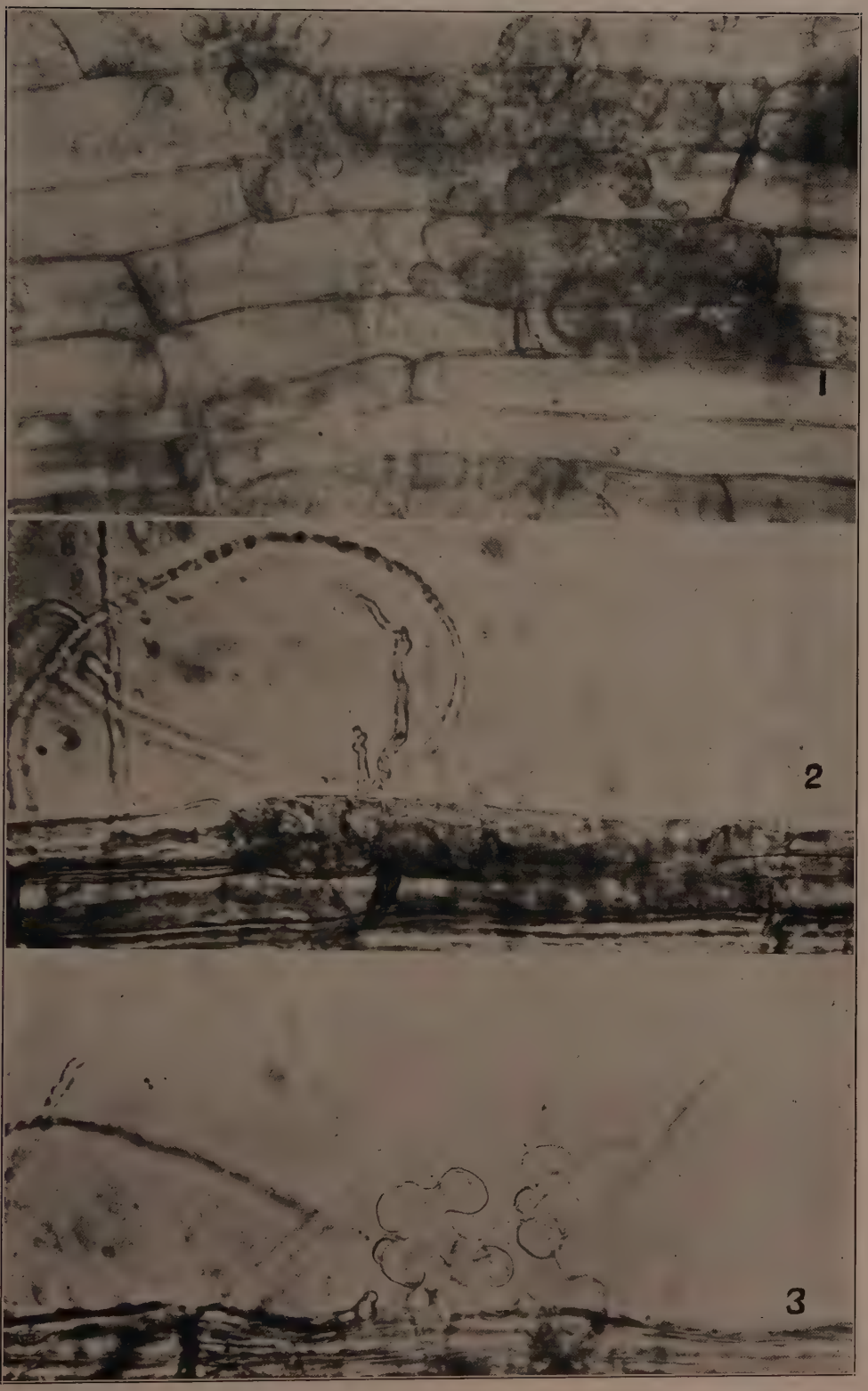
Water culture of *Pythium Butleri* on Lahaina cane root. × 500.

Figure 1—Optical section of young root showing intracellular swollen protoplasmic bodies which are thought to be presporangia.

Figure 2—Hyphae passing from presporangium-like bodies through cell wall to surface of root.

Figure 3—Empty presporangium on surface of root.

PLATE 20.



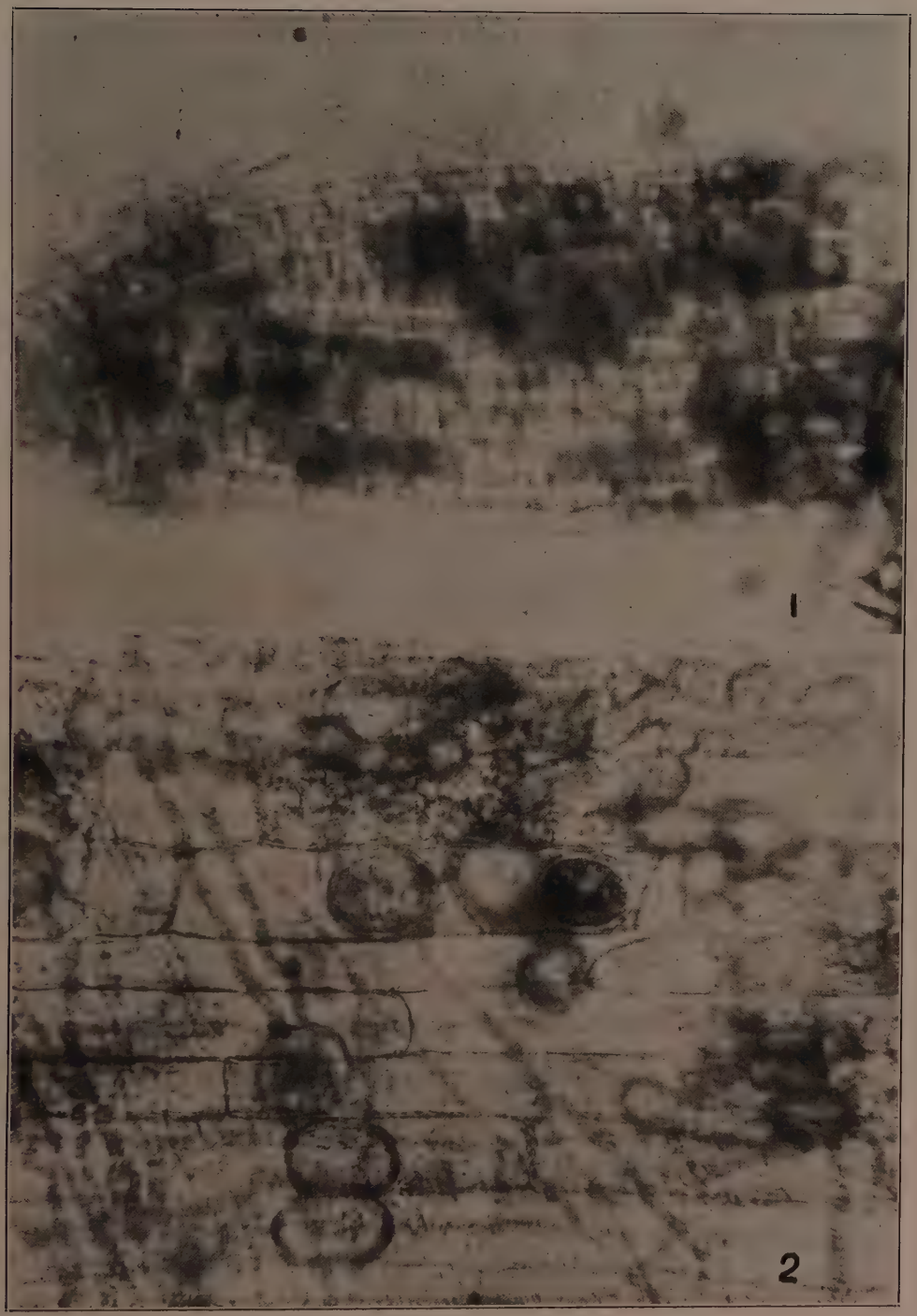
EXPLANATION OF PLATE 21.

Optical section of young cane roots from water cultures of *Pythium Butleri*.

Figure 1—Shadowgraph of infected young root. The dark areas represent the swollen presporangium-like mycelium and oospore content of the cells. × 200.

Figure 2—Closer view of same sort of material. Oospores in the center and presporangium-like swollen mycelium above. × 500.

PLATE 21.



EXPLANATION OF PLATE 22.

The asexual life cycle of a pure culture of *Pythium Butleri* from cane roots, drawn from camera lucida sketches.

Figures 1–4—Presporangia formed at end of hyphae.

Figure 1—Four-day water culture. × 350.

Figures 2–3—Four-day water culture. × 500.

Figure 4—Oat agar culture 13 days and water culture 2 days. × 500.

Figures 5–12—Development of sporangium from the presporangium and maturing of the former. Drawing from sketches made of same sporangium at times indicated. × 500.

Figure 5—February 1, 1921, 11:10 A. M. Streaming of presporangium into vesicle completed. Empty presporangium below.

Figure 6—11:13 A. M.

Figure 7—11:16 A. M.

Figure 8—11:20 A. M. Cleavage beginning.

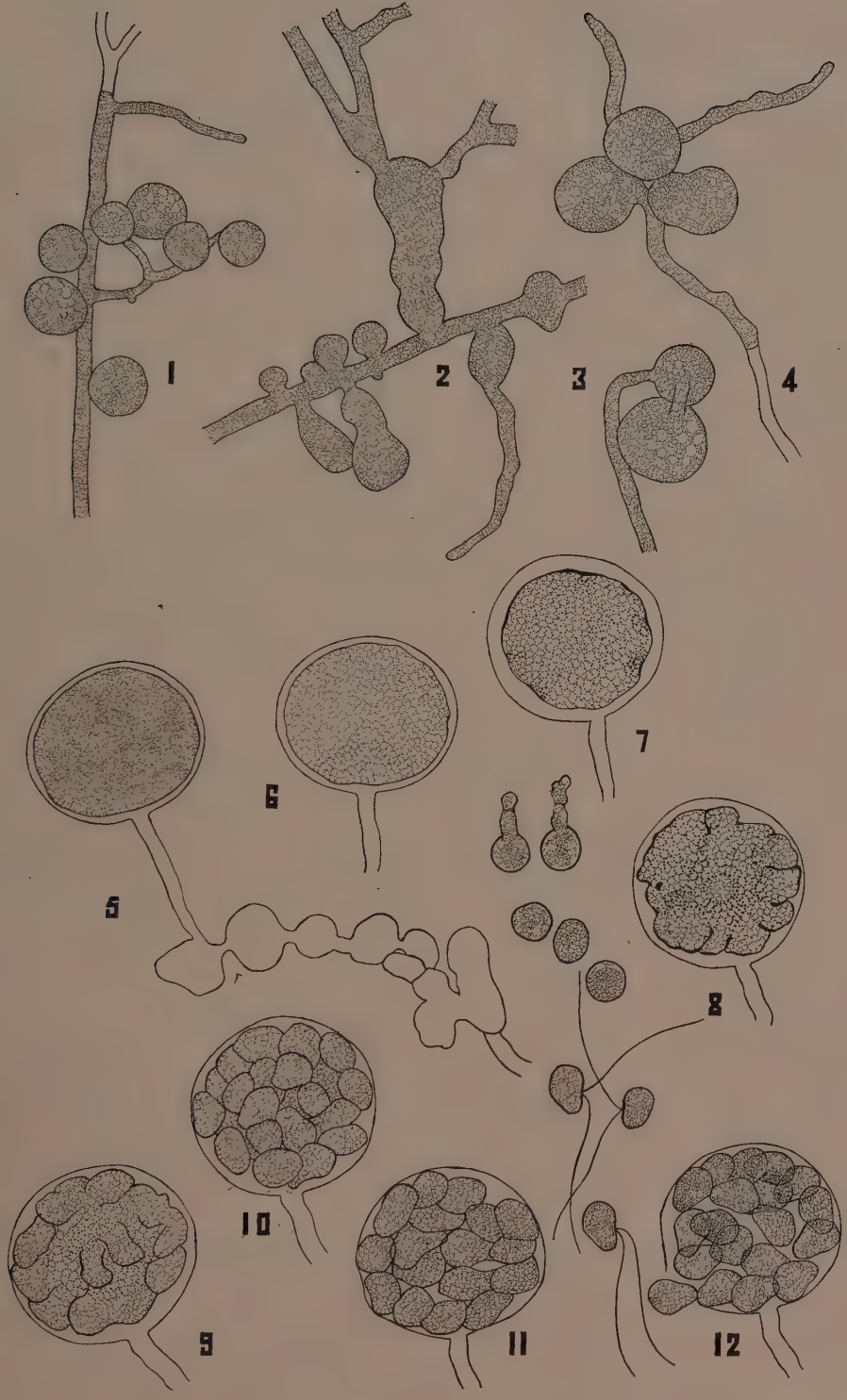
Figure 9—11:23 A. M. Rocking movement of entire content beginning.

Figure 10—11:24 A. M. Rocking of individual zoospores beginning. Differentiation completed.

Figure 11—11:27 A. M. Active, oscillatory individual movement of zoospores present. They ruptured the vesicle and escaped at 11:27 A. M.

Figure 12—The escape of the biciliate zoospores. Above are sketched the zoospores rounding up and germinating after the motile period.

PLATE 22.



EXPLANATION OF PLATE 23.

Morphology of *Pythium spp.*

Figures 1–6—*P. Butleri* from cane. Development of oospores.

Figure 1—Oogonium and antheridia. × 500. From string bean agar culture. Six hours later the oospore was distinctly walled, the appearance being similar to that of figure 5.

Figure 6—Oospore freed from oogonium.

Figures 7–10—*Pythium* sp., the cause of rot of taro. Asexual stage. × 500.

Figures 7–9—Sporangia formed at tips of sporangiophores, which continue growth, pushing spore to one side.

Figure 10—The contents of a sporangium (presporangium) have flowed out through the beak into the vesicle in which the zoospores are differentiated (the true sporangium).

PLATE 23.